Amendments to the Specification

Please amend the specification as indicated below:

Please amend the paragraph on page 50, lines 10 to 31 as follows:

--MAb binding to CCR5+ cells

None of the purified anti-CCR5 mAbs stained the parental L1.2 cell line (data not shown). However, mAbs PA9-PA12 and PA14 stained >90%, and PA8 stained ~70%, of L1.2-CCR5$^+$ cells as determined by flow cytometry, showing they recognized CCR5 (~~Table~~ Figure 1). The anti-CCR5 mAb 2D7, which was a positive control in our experiments, also stained >90% of L1.2-CCR5$^+$ cells. PA8-PA12 and PA14 are all IgG1, and react equally well with a goat anti-mouse IgG, whereas 2D7 is an IgG2a and may react differently with the reporter antibody. Only mean fluorescence intensities (m.f.i.) measured with mAbs PA8-PA12 and PA14 therefore are directly comparable. The rank order of mean fluorescence intensities (m.f.i.) was PA12~ PA11> (2D7=) PA14~ PA10~ PA9> PA8. The difference between PA12 m.f.i. and PA8 m.f.i. was three-fold. Differences in staining intensity between PA8 and the other mAbs remained constant over a wide range of concentrations (data not shown) and probably do not correspond to differences in mAb affinities for CCR5. This implies that PA8 interacts only with a subset of CCR5 molecules present on the surface of L1.2-CCR5$^+$ cells.--

Please amend the paragraph on page 50, line 33 to page 51, line 25 as follows:

--Compared with L1.2-CCR5+ cells, mitogen-stimulated PBMC exhibited different patterns of staining by the anti-CCR5 mAbs. 2D7 and PA14 stained >20%, PA11 and PA12 stained

~10%, PA8, PA9 and PA10 stained <5% of PBMC (~~Table~~ Figure 1). The mean fluorescence intensities of the stained PBMC were about ten-fold lower than those obtained with L1.2-CCR5$^+$ cells for each mAb; their rank order was (2D7>) PA14> PA12~ PA11~ PA10~ PA9~ PA8. Again, this differed somewhat from the order of reactivities observed on CCR5 transfectants. The difference between PA9 m.f.i. and PA14 m.f.i. was seven-fold. Other groups have observed similar differences in the ability of anti-CCR5 mAbs to stain stable, CCR5$^+$ cell lines versus PBMC (28). This may be due to cell-specific differences in CCR5 conformation, post-translational modification or oligomerization. Alternatively, association with other cell surface molecules may differ between cells. Since an obvious choice for such a molecule would be the CD4 cell surface antigen, which is absent from L1.2-CCR5$^+$ cells and present on PBMCs, we also tested the ability PA8-PA12, PA14 and 2D7 to stain HeLa cells transiently expressing CCR5 alone or with CD4. No differences were observed in the ability of any of the mAbs to stain cell surface CCR5 in the presence of CD4 (data not shown). If there is an association between these two proteins, it does not involve epitopes recognized by the anti-CCR5 mAbs available to us. Alternatively, an association between CCR5 and CD4 might only occur on primary lymphocytes.--

Please amend the paragraph on page 51, line 27 to page 52, line 9 as follows:

--Epitope mapping of the mAbs using CCR5 alanine mutants

None of the antibodies were able to detect reduced and denatured CCR5 protein by Western blotting indicating that

they recognize conformationally sensitive epitopes (data not shown). MAb epitope mapping studies were performed using a panel of seventy alanine point mutants of residues in the Nt and ECLs of CCR5. HeLa cells were lipofected with mutant or wild type CCR5 coding sequences appended with C-terminal HA tags, and infected with vTF7 (23) to boost co-receptor expression. The cells were then incubated with the anti-CCR5 mAbs and their binding was revealed by a PE-labeled goat anti-mouse IgG. A second, intracellular stain was performed with a FITC-labeled anti-HA mAb (BabCo). This internal control allowed us to directly normalize staining by the anti-CCR5 mAbs for mutant co-receptor expression levels on the cell surface. Hence, mAb binding to each mutant is expressed as a percentage of binding to wild-type CCR5 (Figure [[1]] 4).--

Please amend the paragraph on page 52, lines 11 to 34 as follows:

--Certain point mutations reduced the binding of all of the antibodies to CCR5 by >50%. In general, PA8-PA12 were the most affected, PA14 and 2D7 the least affected by this class of mutants, which included the cysteine pair C101A and C178A, the Nt mutants Y10A, D11A, K25A, the ECL1 mutant D95A, the ECL2 mutants K171A/E172A, Q188A, K191A/N192A, and the ECL3 mutants F263A and F264A (Fig. [[1]] 4). One interpretation is that these residues are not part of the mAb epitopes *per se*, but that changing them to alanines causes conformational perturbations that have a common effect on binding of all mAbs. We assumed that if a mutation lowered binding of an individual mAb by >75%, and did not also lower binding of most of the other antibodies, the residue was probably a direct contributor to the epitope recognized by the mAb. Using these stringent

guidelines, it was concluded that the seven anti-CCR5 mAbs recognize overlapping but distinct epitopes (Fig. [[1]] 4). MAb PA8 binding to CCR5 depended on N13 and Y15 in the Nt. MAb PA9 and PA10 required D2, Y3, Q4, P8 and N13 in the Nt, and Y176 and T177 in ECL2. MAb PA9 also required S7 in the Nt. MAb PA11 and PA12 binding depended on Q4 in the Nt. PA14 required D2 in the Nt, and R168 and Y176 in ECL2. Finally, mAb 2D7 required Q170 and K171/E172 in ECL2 in order to bind to CCR5.--

Please amend the paragraph on page 53, lines 1 to 30 as follows:

--Chemokine signaling in the presence of anti-CCR5 mAbs

Chemokine receptor-binding agents can be antagonists or, more rarely, agonists of receptor-mediated intracellular signaling. Alternatively, they could have no effect on signaling. CCR5 is able to bind three CC-chemokines, RANTES, MIP-1α and MIP-1β, and transduce a signal that modulates cytosolic calcium levels. We therefore tested the agonist/antagonist activity of various concentrations of mAbs PA8-PA12, PA14 and 2D7. Changes in intracellular calcium concentrations, $(Ca^{2+})i$, were measured in Indo-1-loaded L1.2-CCR5^{+} cells. None of the mAbs stimulated a change in $(Ca^{2+})i$, indicating that they are not agonists for CCR5. PA8-PA12 were also unable to inhibit Ca^{2+} fluxes induced by RANTES (Fig.[[2a]] 5a and data not shown), even at concentrations as high as 100μg/ml, showing they are not antagonists either. These concentrations provide saturating binding of the mAbs to L1.2-CCR5^{+} cells, as shown by flow cytometry and the gp120/CCR5 binding assay (Fig. [[3d]] 6d and data not shown). MAbs PA14 and 2D7, however, blocked calcium mobilization induced by RANTES, although with different potencies (Fig.[[2]] 5a, b). The IC_{50} for PA14

calcium influx inhibition was 50µg/ml, which was approximately 8-fold higher than the IC_{50} for 2D7 (Fig. [[2b]] 5b). RANTES-, MIP-1α- and MIP-1β-induced calcium fluxes were each inhibited by similar concentrations of PA14 (data not shown). None of the mAbs affected SDF-1-induced calcium mobilization in L1.2-$CCR5^+$ cells, which endogenously express CXCR4 (data not shown). Finally, neither mAbs nor CC-chemokines affected cytosolic calcium levels in parental L1.2 cells (data not shown).--

Please amend the paragraph on page 53, line 32 to page 54, line 11 as follows:

--Inhibition of CCR5 co-receptor function by the mAbs

MAbs PA8-PA12 and PA14 were initially selected on the basis of their ability to inhibit HIV-1 envelope-mediated cell-cell fusion. This activity was confirmed and quantified for the purified mAbs. As expected, all six mAbs, as well as mAb 2D7, blocked fusion between $CD4^+CCR5^+$ PM1 cells and HeLa-Env_{JR-FL}^+ cells in the RET assay. The rank order of potency was 2D7~ PA14> PA12> PA11> PA10~ PA9~ PA8 (Fig. [[3a]] 6a). IC_{50} values for PA14 and 2D7 were 1.7µg/ml and 1.6µg/ml respectively, for PA11 and PA12 these were 25.5µg/ml and 10.0µg/ml respectively (~~Table~~ Figure 3). PA8, PA9 and PA10 inhibited fusion by only 10-15% at 300µg/ml. None of the mAbs affected fusion between PM1 cells and HeLa-Env_{LAI}^+ cells, which express the full length envelope protein from an X4 virus (data not shown).--

Please amend the paragraph on page 54, lines 13 to 27 as follows:

--The ability of the different anti-CCR5 mAbs to inhibit entry of a prototypic R5 virus, JR-FL, and a R5X4 virus,

Gun-1, in a single-round of replication, luciferase-based entry assay was also tested. The rank order of potency in the entry assay was similar to the one determined in the cell-cell fusion assay (Fig. [[3b]] 6b). A >50% inhibition of JR-FL or Gun-1 entry with PA8-PA11 was unable to be obtained. The IC_{50} value for PA12 was 2.5 µg/ml. However, inhibition of entry by >60% with this mAb was unable to be obtained. The IC_{50} values for PA14 and 2D7 inhibition of JR-FL entry were determined to be 0.024 and 0.026 µg/ml respectively (~~Table~~ Figure 3), and were 60-fold lower then those obtained in the fusion assay. Entry of dual-tropic Gun-1 was 2-3-fold more sensitive to inhibition by anti-CCR5 mAbs than JR-FL entry (data not shown).--

Please amend the paragraph on page 54, line 29 to page 55, line 5 as follows:

--Anti-co-receptor mAbs might inhibit envelope-mediated fusion either by directly affecting the gp120/CCR5 interaction or by impeding post-binding steps involved in the formation of an active fusion complex. To determine the mechanism of inhibition of viral fusion and entry by PA8-PA12 and PA14, the ability of the different mAbs to block the gp120/CCR5 interaction was tested. For this an assay that detects binding to L1.2-CCR5$^+$ cells of biotinylated HIV-1_{JR-FL} gp120 complexed with sCD4 was used. No binding of biotinylated gp120 was observed in the absence of sCD4 or CCR5, or when HIV-1_{LAI} gp120 was used (Fig. [[3c]] 6c).--

Please amend the paragraph on page 55, lines 7 to 20 as follows:

-- With the exception of PA8, all mAbs abrogated gp120/sCD4 binding to L1.2-CCR5$^+$ (Fig. [[3d]] 6d). Inhibition by PA8 saturated at ~40%, which concurs with flow cytometry data

(~~Table~~ Figure 1) in suggesting that this mAb binds only to a subset of CCR5 molecules on L1.2-CCR5$^+$ cells. MAbs PA9, PA10, PA11 and PA12 inhibited binding with IC_{50} values of 0.24, 0.13, 0.33, 0.24 µg/ml respectively (~~Table~~ Figure 3). Surprisingly, mAbs PA14 and 2D7 were the two least efficient inhibitors of gp120/sCD4 binding, with IC_{50} values of 1.58 and 1.38 µg/ml respectively (~~Table~~ Figure 3). Therefore, there was no correlation between the ability of a mAb to inhibit gp120/CD4/CCR5-mediated membrane fusion and entry and its ability to block gp120/sCD4 binding to the co-receptor.--

Please amend the paragraph on page 56, lines 9 to 24 as follows:

--Combinations of PA12 and 2D7 were the most potently synergistic, with CI values ranging between 0.02 and 0.29, depending on the ratio of the antibodies (Fig. [[4]] 7 and ~~Table~~ Figure 2). The degree of synergy is known to vary with the stoichiometry of the agents. The viral entry and fusion assays were generally consistent in identifying mAb combinations that are highly synergistic, PA12 and 2D7; moderately synergistic, PA12 and PA14; additive, PA11 and PA12; and weakly antagonistic, PA14 and 2D7. The lack of synergy between PA14 and 2D7 is not surprising given that these mAbs cross-compete for binding to CCR5$^+$ cells as determined by flow cytometry (data not shown). The observation of an additive effect of PA11 and PA12 may be an indication that these mAbs bind to slightly different epitopes in CCR5, while sharing a dependency on residue Q4 in the Nt.--

Please amend the paragraph on page 56, lines 26 to 35 as follows:

-- The ability of mAbs PA12, PA14 and 2D7 to synergize with

RANTES in blocking cell-cell fusion was also tested. PA12 and RANTES combinations exhibited moderate synergy (~~Table~~ Figure 2). PA14 and 2D7 exhibited no synergy with RANTES, which is consistent with these mAbs being inhibitory of RANTES binding and signaling (Fig. [[2]] 5a, b). Finally, we tested synergy between mAbs PA12, PA14, 2D7 and CD4-IgG2, which interacts with gp120. We observed moderate synergy between PA12 and CD4-IgG2 but no synergy between PA14 or 2D7 and CD4-IgG2 (~~Table~~ Figure 2).--

Please amend the paragraph on page 82, lines 10 to 25 as follows:

--After the best-fit parameters for K and m are obtained for the experimental agents and their combination, Equation (1) is rearranged to allow for calculation of c for a given f. The resulting table of values (e.g., Figure [[X]] 10) is used to calculate the Combination Index (CI) using the equation

$$CI = c_{1m}/c_1 + c_{2m}/c_2 + c_{1m}c_{2m}/c_1c_2 \quad (2)$$

where

c_1 = concentration of compound 1 when used alone

c_2 = concentration of compound 2 when used alone

c_{1m} = concentration of compound 1 in the mixture

c_{2m} = concentration of compound 2 in the mixture--